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# Gradual Changes in Genetic Based Developments for Phylogenetic Trends in Picornaviruses

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***Authors' contributions***

*This work was carried out in collaboration between both authors. Author IM wrote the review article after consulting the relevant literature and gave the present format while author AS provided necessary guidelines, revised it and finalized it before submission. Both authors agreed for its submission to this journal. Both authors read and approved the final manuscript.*

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## ABSTRACT

On the basis of the several techniques, the new groups and the strains within the Picornaviruses were classified. New groups like human pathogen EV22 (Echovirus 22) was discovered that was found to be highly different from other Picornaviruses to date. The biological properties of Picornaviruses were studied and on the basis of biological properties the viruses were classified into similar groups. The protein composition is rare because most of the copies out of 3 would not be processed to VP2 and VP4. EV23 was said to be similar to EV22 in various sections of genome. The Picornavirus classification is done hierarchically of a family using the quantitative approach with the help of PEDs (pairwise evolutionary distances). Comparison of the GENETIC classification with expert-based Picornavirus taxonomy and the differences in the frameworks were demonstrated, related to the virus groups and genetic diversity that show the classification content and structure. In the GENETIC classification, human Rhinovirus A, human Rhinovirus C and genus Aphthovirus were separated.

*Keywords: Systematics; phylogenetic; genetics; changes; picornavirus.*

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## 1. INTRODUCTION

Picornavirus recombination is a delicate subject as it could occur even within the infected cell. The progeny virus does have a chance to have recombinant viruses if a Picornavirus strain infects the cell. A recombinant virus comprises of genome and part derived from two different virus. This is ssRNA virus approach. There are three types of Poliovirus serotypes and vaccine of oral polio can reduce force of all these three types. Gut cells can be co-infected by the vaccine administration, which leads to the formation of recombinants between serotypes. Presence of wild type Poliovirus can lead to the recombination between vaccine strains and wild type Poliovirus. Polio as a result of recombinants is stated in many documents [1,2].

Reverse genetics in Picornaviruses could help classify them into several groups. If RNA is described in reverse order to DNA then RNA genomes can be manipulated with the same approach as DNA genomes. The function of gene that is in the DNA can be examined if the defined variation can be injected into it. After variation DNA is translated back to RNA and in culture it is transfected into cell. Mutant virus is produced as a result of virus reproduction in that transfected cell. The approach in one experiment with Poliovirus shows that it is important for virion assembly to move forward for VP0 myristylation. Upon the production of genome at VP0 N terminus, the waste of glycine was mutated to alanine [3].

## 2. TECHNIQUES USED FOR CLASSIFICATION AND COMPARISON

### 2.1 Sample Collection

For each sample, human contact, species, and coordinates of Global Positioning System (GPS) were recorded. Soiled materials were acquired only from freshly banked stools. Convenience sample did not have Poliovirus positive faeces. The stool samples were kept in a cold environment to freeze after coming from the field. The temperature at which the faecal samples were placed was minus 80 degree Centigrade and from there they were transported on dry ice to other centres so that they could be analysed [4,5].

### 2.2 Laboratory Testing

Faecal suspension was prepared from each specimen and RNA was extracted. Further tests were carried out for enterovirus (EV), parechovirus (PeV) [6], and sapelovirus using genus-specific TaqMan real-time reverse transcriptase PCR (RT-PCR) assays, that target 5' nontranslated region (5' NTR) [7,8]. Parechovirus assay has the conditions of the reaction regarding the sapelovirus 5= NTR real-time primers (AN626 and AN628) and probe (AN627), annealing temperature of the primers was 58°C instead of 60°C for detection. Sometimes sequencing of 5' NTR real-time PCR amplicon was carried out and BLASTn query of GenBank was used to analyse so that the exclusion of false-positive results probability could be done. The confirmation of the targeted picornavirus genus identity is done as well. Determination of virus identities of genus-specific real-time RT-PCR-positive specimens was done using nested or seminested RT-PCR attacking a region of genome portion that encodes VP1 capsid protein with the next step including sequencing of amplicon [9]. The other human PeV (HPeV) partial VP1 assay was utilized for few specimens that gave sequence that could not be read with the proper VP1 assay. The specimens' cDNA reactions were carried out as mentioned before for parechovirus complete VP1 assay. The reaction of PCR1 is a little different from the parechovirus complete VP1 PCR1 assay mentioned before in that the HPeV primers (AN486 and AN488) were utilized at 0.5 µM in the final reaction volume that is 50 µl.

Like this, the HPeV PCR2 primers (AN268 and AN489) had been utilized at 0.4 µM in the volume of the final reaction was 50 µl. HPeV partial VP1 assay Thermocycler profiles had been mentioned for EV partial VP1 assay [10]. VPl amplicon nucleotide comparison helped find out the virus type identity and deductions were made regarding amino acid sequences using all reference strain's VP1 sequences for single virus genus using script-driven orderly pair type comparison with the help of program Gap (Wisconsin Sequence Analysis Package, version 11.0; Accelrys, Inc., San Diego, CA). The viruses that had rare types (<75% nucleotide similarity with all other types that are known), all VP1 sequences were observed and analysis was done the way it had been mentioned before. Had all the VP1 sequences been different compared to all other types that are known, the sequence would be sent to the *Picornaviridae* Study Group of the International Committee for the Taxonomy of Viruses to get registered as a new type. Pileup program (Wisconsin Package) is utilized for arranging the VP1 nucleic acid sequences and inference of phylogenetic relationships using the

neighbour-joining method used in MEGA, version 4.0 [11], utilizing the Kimura two-parameter technique to analyse evolutionary distances was carried out [12]. The exclusion of portions having arrangement gaps from the analysis was made sure. Estimation of support towards the peculiar tree topologies was done using bootstrap analysis having 1,000 pseudo replicate data sets. Computation of VP1 nucleotide sequence distances for the Picornavirus serotypes was done with the help of multiple detections in NHP. Several other Picornavirus lineages present in a serotype were randomly described as any two viruses (or virus groups) having < 95% VP1 nucleotide similarity with each other, which has been mentioned before for Polioviruses [13,14]. Lineages are highly associated viruses with great similarity and they have a recent common ancestor, whereas viruses having 5% nucleotide differences are said to be genetically apart and away from one another that they could have evolved independently [15].

### 2.3 Nucleotide Sequence Accession Numbers

This study synthesized many VP1 sequences that were stored in GenBank having accession numbers JX538033 to JX538226 (Enteroviruses) as well as JX565593 to JX565644 (Parechoviruses).

### 2.4 Purification and Analysis of Radioactively Labelled Virions

This study used the virus strains that were all from the American Type Culture Collection. The incubation of infected cell cultures for 30 min in methionine-deficient medium was carried out to label cells with [35S] methionine and this medium's place was taken by medium having [35S] methionine (Amersham; 50uCi/ml). After the cytopathic effects that appeared, harvesting of the cells was done and the cells were allowed to release virus particles using three cycles of freezing and thawing. Centrifugation cleared out cell debris and purification of the virions was done using polyethylene glycol-NaCl precipitation and sucrose gradient centrifugation [16]. Sodium dodecyl sulfate-polyacrylamide gel electro-phoresis was used to study and examine the purified viruses and infected cells [1,17].

### 2.5 Amino Acid Sequencing of Capsid Proteins

Micro preparative SDS-PAGE along with an apparatus mentioned earlier [18] was used to take out the capsid polypeptides from purified virus of about 50 µg [19]. Utilization of SDS-polyacrylamide tube gel (4% stacking and 11% separation gel; 0.3 by 8.5 cm) was done, and electrophoresis was carried out at 130 V overnight. The lower part of the tube gel was from where the proteins were taken into 10 mM ammonium bicarbonate that rushed (50 j,i/min) from within the cell (100, µl) present near the lower part of the tube gel and was parted with the help of dialysis membrane from the lower electrode buffer. Portions (1 ml) were gathered and analysed again (1/10 of the fraction) for the eluted proteins localization. The two portions having 38-kDa polypeptide were joined and concentrated to 500 µl using a vacuum centrifuge and this was done before the cleavage of the protein using 4% (wt/wt) N-tosyl-L-phenylalanine chloromethyl ketone (TPCK)-trypsin (Sigma T-8642) around 37°C for almost 5 hours. The peptides at the end were taken out using reversed-phase chromatography on a Rexchrom Prep-5/300 C4 FEC column (0.3 by 15 cm) (Regis Chemical Company) along with a linear gradient of acetonitrile (3 to 60% in 60 min) in 0.1% trifluoroacetic acid having a flow rate of about 0.5 ml/min. A tiny anion exchange column (0.21- by 3-cm) [Fractigel TSK DEAE-650 (M); Merck] was utilized next to the reversed-phase column so that SDS intrusion could be evaded using the reversed-phase separation. Physically the Eluted peptides were gathered, and vacuum centrifuge was used to concentrate them [20]. The gas-pulsed liquid sequencer was used for examining the sequence of the peptides.

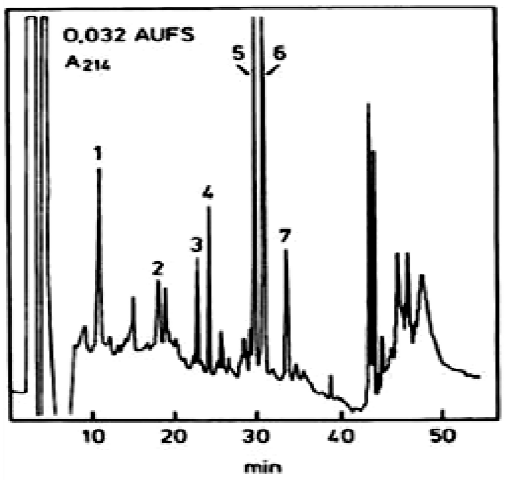


**Fig. 1. Reversed phase chromatography of tryptic peptides from largest EV22 capsid protein (38 kDa) [21]**

### 2.6 Analysis of Acid Liability

EV11, EV22, EV23, Poliovirus type 1, human Rhinovirus type 1B (HRV1B) and HRV14 were the strains for which the acid liability tests were run. The cells that were susceptible provided the medium for the viruses to grow and harvesting was done after the completion of the cytopathic effect. Pelleting of cell debris was done and assay used the supernatant. Before the 1 hour incubation at 37 C, equal volume of citric acid buffer (pH 4) was poured into supernatant. Neutralization of mixture was done and titration was carried out to estimate the infectivity within the appropriate cell lines. Comparison of titers with the untreated supernatants was done to calculate acid liability [21, Fig. 1].

### 2.7 Determination of Density of Virus Particles

EV11, EV22, and HRV14 virions' (labelled as well as purified as mentioned earlier) buoyant density was calculated using CsCl gradients. Resuspension of purified virions was done in CsCl (1.35 g/cm3 in 100 mM Tris-HCI, pH 7.5) and preceded for about a day at 22°C within a Beckman SWSSTi rotor (45,000 rpm). 250-,ul radioactivity aliquots, cloning of cDNA and sequencing were done using EV23 RNA (1 ug) that was reverse transcribed and the homopolymer was tailed with C residues and it's cloning was done into G-tailed pBluescript vector using the cDNA-RNA hybrid procedure [22]. A cDNA clone sequencing was done using the dideoxynucleotide method. Peptide inhibition of binding of the cell surface receptor. Preincubation of A549 cells having confluent cell monolayers with or with no peptides (RRRGDL or RRGEL) for about 45 minutes at room temperature. Peptide solution was taken out and infection of cells with purified viruses with or without the peptide. After incubating it for 15 minutes at r.t.p, inoculum virus that is not absorbed was washed out with the help of plaque overlay having 0.5% carboxymethyl cellulose poured into the culture medium. The calculation of the results was done after 2 or 4 days and the plaque number was observed [23].

### 2.8 Computer Analysis

The arrangements of the amino acid sequence were done with the help of Genetics Computer Group software [24].

## 3. CLASSIFICATION OF PICORNA-VIRUSES

The International Study Group put forth the following subgroups of picornaviruses where major groups were two; Picornaviruses of human origin having Enteroviruses with Polioviruses, Coxsackieviruses A, Coxsackieviruses B and Echoviruses, Rhinoviruses and unclassified subgroups and Picornaviruses of lower animals. Picornaviruses have five genera (Aphtho-, Cardio-, Entero-, Hepato-, and Rhinoviruses), based on physicochemical traits and pathogenicity but advancements also include molecular properties [24,25].

### 3.1 EV22

EV22 has similar physical traits prevalent in Enteroviruses like pH stability and buoyant density, but it has different molecular properties [26-29]. There is another strain in the group that was discovered and it is EV23. The EV22 buoyant density matches the Picornaviruses, especially the Enteroviruses. EV22 also has acid stability which is a property present in Picornaviridae members. The 2 differences are based on VPO protein. EV22 has three capsid proteins. VPO is not cut in mature EV22 virion. Some Picornaviruses have few unprocessed VPO particles inside them, the cutting process in most of the VPO polypeptides is considered necessary for alterations that are linked to the maturation and stabilization of the virus before escaping. This is a chief difference from other Picornaviruses. VP1 proteins arrangement is supported by EV23 sequence data that represents amino acid similarity of EV22 protein. Many differences among the viruses are present in the loops linking that is predicted, beta sheets, specifically B-C, E-F, and G-H, that are chief immunogens in other Picornaviruses [30-32]. The diversity of the C-terminal region is great between EV22 and EV23, showing that it might be an immunogenic site. CAV9 isolate has a working RGD sequence; this motif is located in VP1 analogous to EV22. RGD motif is present in G-H loop in FMDV, a chief immunogenic site and a region of high variability between serotypes [2].

In conclusion, EV22 and clearly EV23 represent a new Picornavirus group with characteristics partly found only in EV22 and EV23. Sequence results have shown that pathogen Echovirus 22 (EV22) is different from all the other Picornaviruses studied to date and this difference is based on genetics. The configuration of protein

is rare as one out of three main capsid proteins, VPO, the processing to VP2 and VP4 does not occur which is often seen when the virus is maturing and it is present in other Picornaviruses. EV23 had many similarities with EV22 in various genomic regions [21, Fig. 1].

### 3.2 Polio, Coxsackie and Echoviruses

The first serotypes were taken from the monkeys through intracerebral inoculation. These three viruses caused poliomyelitis in man. The infant mouse was the host of the virus that was inoculated and large unknown Picornaviruses group was discovered. They were called Coxsackie viruses [33] in 1949 and further classified [34] into serotypes that cause myositis in infant mice (labelled group A), and some caused focal myositis as well as lesions in other organs (labelled group B). These two groups contain 30 serotypes. Few group A serotypes Coxsackieviruses [35] caused herpangina and other members of group B caused epidemic myalgia or pleuro-dynia (Bornholm disease) [36]. Some thought these effects were of poliomyelitis virus type 4. ECHO viruses were the new group and were discovered in 1955 by a committee of American workers using the cell culture techniques. The criteria used to make it a part of the Picornavirus group is (i) that the virus should be cytopathogenic to the primate cell cultures and not infectious to the infant mice; (ii) it should be take from the human alimentary tract and infect man; and (iii) that it should not be associated with other groups of viruses taken from the human alimentary tract. At first there was no method for Echoviruses in terms of size, ether resistance, or type of nucleic acid-the three essential requirements for -a Picornavirus. Echovirus type 10, was named Echovirus and is not a part of picornavirus group [37]. Echovirus type 10 is Reovirus type 1 now in terms of its larger size and other significant biological properties that differentiate it from the other Echoviruses. By using cell culture techniques to extract viruses from the alimentary canal of humans it was evident that some viruses having Picornavirus properties were not to be placed in to category of poliomyelitis virus, Coxsackievirus, or Echovirus. Therefore Echovirus type 9 strains were discovered after subculturing in cell culture to make lesions in infant mice like the ones made by group A Coxsackieviruses [38].

### 3.3 Proposal of the Term "Enterovirus"

The Poliomyelitis viruses, Coxsackieviruses and Echoviruses had similar traits of size, resistance to ethyl ether, and origin in the human alimentary tract. In 1957, committee put forth a proposition that the poliomyelitis viruses [39], the Coxsackieviruses, and the Echoviruses are to be placed in one family; Enteroviruses. Enterovirus named for the viruses recovered from the human alimentary tract. It was later said that such viruses had to cause an infection in the alimentary tract [40]. In 1962, the same committee put forth another proposition of a single numbering system for the Enteroviruses [41] because the boundary lines within the prior subgroups were indistinct and some strains looked like they were linked to one subgroup and other strains of the same immunological type looked like they belonged to some other group. Subgroups close relationships were elaborated by cross complement-fixation tests with paired human sera [42-44]. Different Enteroviruses and other viruses could be responsible for the same syndrome, and same Enterovirus could be responsible for single syndrome so pathogenicity can be used in labs [24,32].

### 3.4 Proposal of the Term "Rhinovirus"

In 1960 the common cold viruses were discovered by the workers working at the Common Cold Research Unit located at Salisbury, England [45-48]. They were previously known as "Salisbury strains" [49] and finally they were named [30,50] "Rhinoviruses". They were similar to Enteroviruses in many ways, like Size, resistance to ether and kind of cytopathic effect it caused in the cell culture. They were varied from Enteroviruses in that they reproduced in the nose and not in stools, and needed special cell culture conditions (incubation temperature=33 C, pH approximately neutral and culture rotation) for their separation. The Rhinoviruses have two groups -the "H" strains that only propagated in cell cultures of human and the "M" strains that grew in monkey cell cultures. Certain resemblance of "M" strains of rhinoviruses with some viruses was indicated (JH and 2060) that were taken out from the mild acute respiratory disease [51,52]. The viruses were labelled as strains of Echovirus type 28 [53]. Echovirus type 28 grew well if cultures were rotated and pH was kept near neutral range [7.2 to 7.0; 30, 57]. Two M strains (B632) demonstrated by Salisbury group is closely linked to Echovirus type 28 with respect to the antigenic properties and it probably might be placed as the strain of the latter virus [54-55]. Using the cell culture conditions that were first demonstrated by the investigators at Salisbury, but some other kind of

cell culture, some other workers separated a number of viruses from "common colds," which were named "Coryzaviruses". It was still not known as to why the new group was to be made. Now it is believed that there are no differences between such viruses [56]. One investigator who separated Echovirus type 28 said that Enteroviruses should be called "Respiroviruses". He also proposed that Echovirus type 28 be labelled type 1 of a new virus group which is "Muriviruses" [55] (Mild upper Respiratory infection viruses). It was studied that Rhinoviruses reproduce in the nose still no data was put forth to support the proposition.

### 3.5 Foot and Mouth Disease Virus (FMDV)

Antigenic variations in FMDV were of substantial use, indicating mutations and emergence of new strains that could add up to the classification system [57,58]. Mutation rates in RNA viruses and FMDV is high, ranging from $10^{-3}$ to $10^{-5}$ per nucleotide site per genome replication, and this is because of the absence of error correction mechanisms while the RNA is being replicated [59]. This elevated error rate causes variations in FMDV replicated genomes and they differ from the original parental genome having 0.1 to 10 base positions, Quasispecies concept was given forth to elaborate the effects of errors during replication on the replicating RNA particles evolution. Genome sequences differ in all viruses and selection is done at population level instead of the individual level. Therefore the “wild type” is absent as such but an “average” phenotype exists that was taken up and replicates in a better way in the present environment. The variation in phenotype of viruses occurs when the codon changes due to a certain mutation. Genome has a region known as capsid-coding where the maximum change occurs and as a result create changes in antigenicity. FMDV has been revealed and RNA recombination was examined in tissue culture within genome coding regions for NS proteins. P1 region in the capsid-coding may play its part in the change that occur in genetics in FDMVs that is separated from the field [60-62]. Furthermore, if in tissue culture immunologic pressure doesn’t exist; antigenic variation can be found in FDMV showing the involvement of antigenic sites on the virion in virus physiology. The surface antigenic sites of FMD virion have been seen in five of the seven serotypes of the virus [63]. Identified sites of antigens were four in minimum, including one or more of the capsid proteins, VP [1, 2, and 3]; still it is also possible that all four sites are not contained by every single serotype. It is of great interest that viral protein sheets are connected with flexible loops which contains elements of three sites, and VP1 C terminus is contained by at least two of those sites. The major site of antigen exists in G-H VP1 loop, it is considered as hub for all serotypes and the safest response is also transmitted to it. RGD receptor binding recognition sequence is also contained by this antigenic site. The variations are bounded to the defined viral surface regions. Even the immunologic pressure might be produced by the best vaccine within the population which leads to the advent of new variant [64,65].

Reemergence of FMD in developed countries indicated the strain resistance. Taiwan which had been a FMD free country for 68 years since 1997 was hit by a devastating outbreak of the disease. The outbreak occurred in March 1997, affecting and killing more than 4 million swines. The virus identified as the causative agent was O/taw/97. Many pigs were slaughtered and different vaccinations were launched to contain the disease. When experimental studies was done at world reference laboratory for FMD at Pilbright, the result showed that there was no involvement in infection bovine tissue culture cells by the virus isolated from infected pigs. Further studies by Mason and his colleagues on the virus revealed that the factor due to which the growth of this virus is restricted in bovine cells (in vitro and vivo), is the presence of viral NS protien 3A. This epidemic outbreak also hit East Asia, South Africa and UK. The causative agent for these outbreaks was identified by The World Reference Laboratory as a serotype O PanAsia lineage virus which spread through the Middle East, Turkey, and Eastern Europe, and then targeted China, South Korea, Japan, Mongolia, and far-east Russia. Detailed genomic analysis of the virus revealed a distinct difference between virus O/taw/99 and O/taw/97 on the basis of their nucleotide sequences. But virus spreading in Middle East and India were closely related to O/taw/99. O serotype O/SKR/2000 virus was identified in infected cows by world reference laboratory which also resembled O/taw/99. This virus, O/JPN/2000 was placed in the same lineage having O/TAW/99 and O/SKR/2000 using the sequence analysis. PanAsian type O lineage resembled O/SKR/2000 as well as O/JPN/ 2000, a serotype not known in South Africa [1,64].

## 4. GENETIC CLASSIFICATION VS ICTV TAXONOMY

DEmARC is a quantitative method used for classifying the virus family hierarchically on the basis of intervirus genetic divergence. Its evaluation has been thorough in terms of consistency and stability keeping in view the chief parameters like the amount and diversity of the input data, the alignment construction method, and the calculation of intervirus divergence. DEmARC-mediated Picornavirus classification is now called GENETIC classification.

### 4.1 Genetic Classification versus ICTV Taxonomy: Species Level

In taxonomy, at species level which is the principal level, the genetic classification consists of 38 clusters. Twenty seven out of these thirty eight correspond to twenty seven species of the ICTV taxonomy one to one, three surrounds a single species (Human Rhinovirus C: HRV-C), and other eight includes recently revealed viruses that in the start of study were not properly classified. HRV-C is further divided into three clusters provisionally called HRV-Cα, HRV-Cβ, and HRV-Cγ. The twenty seven that are in contact with acknowledged species that consist of past classified and newly discovered viruses, counting simian enteroviruses getting united with Human enterovirus A and B respectively. *Scaffold* makes group with *Theilovirus* [67], *Possum enterovirus* with *Bovine enterovirus*, and *Porcine Kobuvirus* with *Bovine Kobuvirus*. After the virus was updated, excluding *Theilovirus* expansion in the host range of other species occurred. Three families and two genera excluding *Picornaviridae* fresh RNA phylogenetic study discovered that virus species exchanging host more quickly than they used to do in past. Novel viruses are Cosaviruses (4 clusters; CosaV-A, CosaV-B, CosaV-C, CosaV-D) [68], seal Picornavirus (1 cluster; AqV-A), human klasse- and Saliviruses (1 cluster; SaliV-A) [34], Rhinoviruses related to but segregated from *Human Rhinovirus A*, HRV-A (1 cluster; *Human Rhinovirus A*β, HRV-Aβ) [69,70] and simian Enteroviruses not part of *Simian Enterovirus A* (1 cluster; SiEV-B) [71]. Thirty-two sequences from a total of 38 species have sequences more than single sequence. Some of them estimate the PED range of the 38 species clusters, and this is called “interspecies” genetic divergence. The 38 species had variable virus sampling in terms of a range of 1 (six species) to 260 (FMDV) sequences. The relevant intragroup PED ranges varied 10-fold between the species; having more than one different sequences, and maximum altering from 0.04 (AvEMV) to 0.41 (HRV-A). All clusters were finished apart from the three species clusters [42]. The viruses in the three species clusters that were not completed belonged to HRV-A (total 96 viruses and 14 viruses made pairs with larger-than-threshold distances), *Bovine kobuvirus* and the given species-like cluster HRV-Cγ [72]. Within such species, respectively, 3.6%, 16.7% and 50% of intragroup PEDs were more than the species threshold. In all, they make less than 0.19% (175 out of 93,857) of the whole intragroup PED values at this state. *Bovine Kobuvirus was divided into* two clusters that examine the threshold and in the analysis of three data sets are host restricted [73,74].

### 4.2 Genetic Classification versus ICTV Taxonomy: Rhinoviruses

Why there is a great change between Genetics classification and ICTV taxonomy in considerations of HRV-C though supporting all other species virus composition? A vital role could be played particularly by both HRV-C progress and the two characterization concepts. Genetically there is great change in capsid (1A, also have other name e.g. 1D proteins and VP4) and non-structural (3D) regions, it was previously reported that diversity in these two were more than those of other Rhinoviruses [40]. This variance is considered smallest in the 1D proteins and whole HRV-C versatility was considered below the diversity range of the species, which helps HRV-C to be considered as a single species. This way only major capsid proteins were used in cluster that is part of DEmAR mediated classification; similarly it is observed that a single species is formed by HRV-C viruses [66, Fig. 2]. However, examining the data set that consist of six family wide preserved proteins. Change in the HRV-C is comparatively more than its most diverged HRV-Cγ (subset of HRV-C viruses), it also exceeds family wide differentiated limit values: 0.424, 0.392, and 0.37 respectively. Most probably it was due to the combined effect of well-matched structural and non-structural phylogenetic signals. Change in HRV-C is too large, it exceed more than half of HRV-Cγ intragroup distance. It is noted that HRV-Cγ support is overall the lowest and one of those three which is below 100%. This is very unusual that the sampling of virus in this interim species along with other two

sister taxa of HRV-C is very limited. So it can be believed that in future by adding up sequenced genomes, HRV- Cγ can be further divided and HRV-C will be four times more in number as compared to today. Every single species communicates to different and major HRV-C phylogeny's family [75]. In addition, the Genetic classification suggests and acknowledges HRV-Aβ which is potentially a new Rhinovirus species. It is made up of three viruses and lies in the recently acknowledged clade D Rhinoviruses [69]. On the whole, it is discovered that there are at least 6 Rhinovirus species instead of 3. Examining human Rhinovirus complex structure could lead to exploration of molecular basis of the existing clinical heterogeneity of Rhinovirus infections in human.

### 4.3 Genetic Classification versus ICTV Taxonomy: Genus Level

A genus level that is included in the genetic classification has 16 clusters, 11 ICTV genera, 2 single genuses and 3 recently discovered viruses. The genus Aphtovirus was divided into two groups, single species (Equine Rhinitis A virus) and two species (Bovine rhinitis B virus) and foot and mouth diseases [64]. The minimum PED between the two groups of virus is 1.03 abed is significantly greater than 0.905 that is distance threshold of the genus and they are almost similar to the other sister genera virus pairs i.e., *Enterovirus* and *Sapelovirus* or *Senecavirus* and *Cardiovirus*. Looking at the range this group can be fit in supergenus which is explained below. This result was also replicated in organizations of two estimated data sets where these viruses exist but be at variance to genome region and virus selection [66, Fig. 2]. It is noted that monophyletic virus group associated Papian like fold and proteloyic activity can change an L protein and could be defined as larger group molecular marker of which sister genus Erbovirus is also a part [71]. So in future dividing genus Aphthovirus into two genera can be greatly supported by reviews of taxonomy. Recently discovered viruses formed three genus clusters which consist of four species of Crotaviruses, one species of seal Picornavirus, and one species of Saliviruses. With omission of Enterovirus all genus groups got completed. As a result, the intragroup PED value crosses the genus is less than 0.02% (21/152194). Out of 16 genera seven are non-singleton. Some defines the specified PED range of genus, known as "interspecies intravenous" genetic disparity.

### 4.4 Genetic Classification versus ICTV Taxonomy: Recognition of the New Hierarchical Level Super Genus

An additional rank is recognized by the GENETIC classification known as supergenus. In virus taxonomy it has no equal and this level has complete and strongest support [66, Fig. 2], showing that it may reproduce a grouping that is inherently and evolutionary sensible. At this very stage five non-singleton supergenera that comprise of more than one genus. The proportion of species to genera in a virus starts from 28:10. Within these supergenera, four exemplified unions between Enterovirus and Sapelovirus, Cardiovirus and Senecavirus, Hepatovirus and Termovirus, and newly discovered Saliviruses with Kobuvirus. The last one in the ICTV taxonomy makes link with the genus Aphthovirus. Which in the Genetic classification is then divided into two genera? Singleton genera with other 10 species reside in other six supergenera, only one ICTV resides in four supergenera, whereas two recently discovered viruses titled as Coasviruses and seal Picornavirus are made up of other two supergenera. All supergenus groups are through with the omission of the Enterovirus/Sapelovirus union. As a result, the intragroup PED value crosses the supergenus is less than 0.25% (7/2814). Particular supergenus PED range is determined by five non-singleton supergenera, which is defined as "interspecies intergenus intrasupergenus" genetic disparity.

### 4.5 Multimodality of PED Distribution and Evolution of Picornaviruses

The PED incoherence existence is explained and defined by the virus speciation model. Where threshold of species exists and if the threshold is crossed it will likely bound intergroup genetic disparity but not intragroup one. This incoherence describes that change in birth and death rate is of large scale and might have happened through all virus families [76]. If hosts are followed by Picornaviruses, then virus speciation and extinction will mainly produce a periodic pattern in PED distribution represented by changing peaks and valleys. This study observed that in the Picornaviruses evolution two waves of extinction will separate two main waves of speciation at genus and supergenus levels. Which probably is describing the environmental change [77].

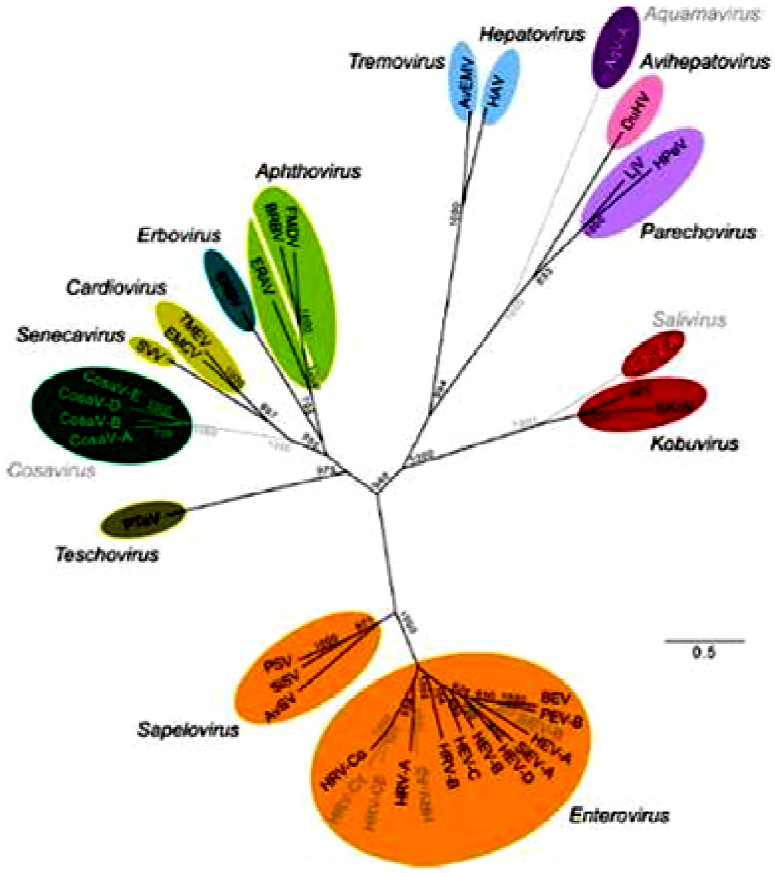


**Fig. 2. Phylogeny and genetic classification of Picornaviridae [66]**

### 4.6 Genetic Classification and Taxonomy of Picornaviruses: Two Different Perspectives

As discussed earlier, only few taxa were recognized at the genus and species level of Picornaviridae, where ICTV taxonomy and genetic classification have striking agreement. The match found is of less importance [78], since the fundamental decision making concept seem to fulfil different criteria. Two concepts which are either exclusively or predominantly genetic based can make this criteria clear e.g. DEmARC or ICTV. The diversity of virus and its effect on partitioning are characterized, which in virology is the initial target and a vital subject of research. In Fig. 3 virus family is characterized and graphically (circular diagram) represented. It illustrates that by characterization the distributions of the intervirus genetic disparity is partitioned and not portioned, respectively [2,79]. The PED range detected in the family defines radius of the circle, there is a linear increase in the intervirus genetic disparity from zero to the maximum observed PED (centre of circle). The radial dimension boxes (Taxa) correspond to PED range of several characterized level. Genus layer follows the most external layer species, whereas supergenus layer nesting nearest toward the centre of circle. In Fig. 3 scheme of bright and soft colours are respectively used to classify that if PED range within each taxon has been sampled or not sampled. The white colour at the circle inner part shows that PED range has not yet been partitioned. To give a balanced genetic foundation comparison of both concept involving maximum possible taxa, Genetic classification is to be followed by the ICTV taxonomy (Fig. 3) and accept all taxa accommodating new viruses and differently classified Aphthovirus and Human Rhinovirus. This result in a match between Genetic classification and the taxonomy with respect to the virus sampling per taxon (the most outer layer), genus structure and species. At the level of species, a definite genus differentiation

criterion is applied by PSG between all varieties of genus species. As a result, the species intragroup genetic disparity limitation varies too much among genera. Consequently, Fig. 3 A compares and shows that heights of same genus are equal. At the level of genus, differentiation criteria for maximum qualification intragroup genetic disparity are not given and every genus is differentiated individually, generally by source of standard analysis of phylogenetic relationship. To reproduce this method, an observed intragroup genetic disparity is represented in Fig. 3 A. Also Fig. 3 A taxon 15.1 shows that height of genera that has a single species is nil. In comparison, Fig. 3B shows that heights of species, supergenus taxa or genus are standardize and specified to certain level. As in this concept family wide boundaries on intragroup genetic disparity are arranged. As a result of the implementation of family wide differentiation thresholds, size of the white area in ICTV partition that is large part of the PED space is compared with the DEmARC.in addition to this, DEmARC loosens the intragroup genetic disparity ranges that is described for most taxa, showed in soft colours in Fig. 3B. Concept of ICTV doesn't provide such forecasts. The white central area in Fig. 3A and 3B also make it clear those most unsociable viruses' relations in the Picornavirdae remains totally amorphous. In the concept of DEmARC, as the area is to some extent partitioned by super genera so it is small. With the introduction of sub family it could be further partitioned. Genera have maximum heights and represented as boxes [66, Fig. 2].

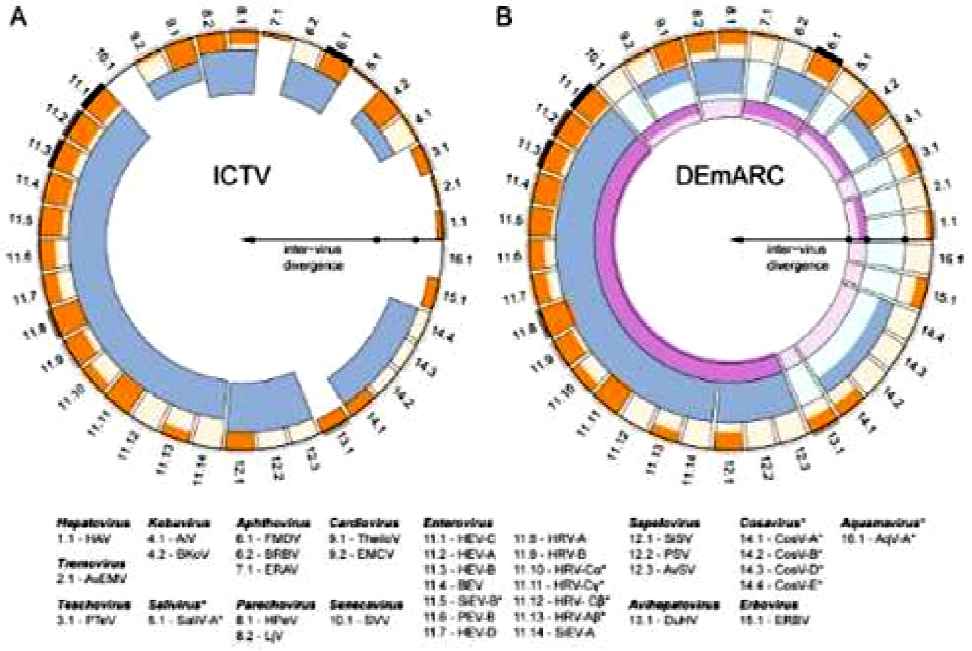


**Fig. 3. Taxonomy diagrams and comparison of classification frameworks under the ICTV [66]**

**Table 1. Physicochemical and biological properties of EV22 compared with those of existing picornavirus groups [21]**

| Picornavirus group | Density ($g/cm^3$) or range | pH stability | Poly (C) | 5' UTR group | Funtional RGD | Shutoff | Myristoylation | VP0 cleavage |
|---|---|---|---|---|---|---|---|---|
| EV22 | 1.36 | Yes | No | 1 | Yes | No | No | No |
| Enteroviruses | 1.34 | Yes | No | 2 | No | Yes | Yes | Yes |
| Rhinoviruses | 1.39-1.42 | No | No | 2 | No | Yes | Yes | Yes |
| Aphthoviruses | 1.43-1.45 | No | Yes | 1 | Yes | Yes | Yes | Yes |
| Cardio viruses | 1.34 | Yes | Yes | 1 | No | No | Yes | Yes |
| Hepatoviruses | 1.34 | Yes | No | 1 | No | No | No | Yes |

## 5. CONCLUSION

Several techniques like sample collection, laboratory testing using genus-specific TaqMan real-time reverse transcriptase PCR (RT-PCR) assays, nucleotide sequence accession numbers, purification and analysis of radioactively labelled virions, amino acid sequencing of capsid proteins, analysis of acid liability, determination of the density of virus particles including cDNA clone sequencing and computer analysis were used to determine the properties of various viruses of Picornavirus and comparisons of the viruses on the basis of these properties were made to classify the viruses within Picornavirus. The amino acid sequencing of capsid proteins was done to compare and to see the relationship between the strains of Picornaviruses. The classification done classified the Picornaviruses into Picornaviruses of human origin (Polioviruses, Coxsackieviruses A, Coxsackieviruses B, Echoviruses, Rhinoviruses, Unclassified) and Picornaviruses of lower animals. The genera of Picornaviruses were 5; (Aphtho-, Cardio-, Entero-, Hepato-, and Rhinoviruses). The examples include Polio, Coxsackie, Echoviruses, and FMDV etc. The new group EV22 was compared with Enteroviruses, Rhinoviruses, Aphthoviruses, Cardioviruses, Hepatoviruses for classification of Picornaviruses. This comparison for classification is mentioned in Table 1 above.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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